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Design of novel antifungal mucoadhesive films Part II. Formulation and in vitro biopharmaceutical evaluation

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Abstract

This paper deals with the formulation of the mucoadhesive films containing nystatin. The design and formulation of the films were based on the mucoadhesive properties of carbomer 934P (CB) and carboxymethycellulose (NaCMC), and also on the plasticizer properties of polyethyleneglycol 400 (PEG400). A surfactant (ascorbyl palmitate, ASC16) was added to the system to aid in nystatin dispersion. Addition of these last two components produced a significant improvement in physical–mechanical properties (flexibility and strength) as well as an increase in the nystatin release rate. X-ray powder diffraction (XRPD) and scanning electronic microscopy (SEM) were used to evaluate the morphological changes in the films while PEG400 and ASC16 were added to the formulations. Furthermore, the in vitro nystatin profile release was determined.
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Keywords: Mucoadhesive films; Nystatin; Ascorbyl palmitate; PEG400

1. Introduction

The antifungal drug nystatin is widely used against oral and gastrointestinal mycoses. However, the efficacy of conventional dosage forms for use in the oral cavity is limited by the short residence time of the formulation (Millns and Martin, 1996; Weatherell et al., 1996; Machida and Nagai, 1999). Mucoadhesive gels, tablets and films have been designed to overcome these shortcomings. Tablets and films have given better performances than gels mainly due to the short residence time of gels in the oral cavity over long periods of time. On the other hand, mucoadhesive flexible films appear to be more suitable than tablets owing to their better adaptation to the mucosal surface. An ideal buccal film should be flexible, elastic, and soft but adequately resistant in order to prevent breakage due to stress from oral activities. Moreover, it must also possess good bioadhesive strength so that it can be retained in the mouth for long periods (Hoogstraate and Wertz, 1998; Peh and Wong, 1999; Lee et al., 2000). In earlier works (Llabot et al., 2002, 2004), we have designed and formulated mucoadhesive dosage forms for local administration of nystatin in the oral cavity. These formulations consisted of tablets that showed good "in vitro" mucoadhesion and release characteristics. However, as previously mentioned, the flexibility of films could be the main comparative advantage. Therefore, we have formulated mucoadhesive films using hydrophilic polymers (CB-NaCMC) and plasticizer (PEG400). Also, with the aim of facilitating the incorporation of nystatin into the system, a surfactant (ASC16) was added to the formulation. The films were obtained by means of a casting process of the aqueous dispersions of the components. In the first part of our research we studied some properties of the film forming gel both before and during the casting process, such as morphological concerns and rheological behavior (Llabot et al., 2006). Now, in this second part of our work, we wanted to investigate the pharmaceutical behavior of the film as a drug delivery system for buccal application of nystatin. Consequently, the "in vitro" mucoadhesive properties, the influence of the ingredients (plasticizer and surfactant) on the system performance, morphological aspects, type of dispersion and drug release were all evaluated.

2. Materials and methods

2.1. Materials

Nystatin USP (Parafarm, Buenos Aires, Argentina), Carbomer 934P (Acritamer 934, a gift from RITA Corporation,

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Table 1
Composition of mucoadhesive films

Film	CB (g)	NaCMC (g)	Nystatin (g)	PEG400 (ml)	ASC16 (g)
A	0.25	0.25	–	–	–
B	0.25	0.25	0.2	–	–
C	0.25	0.25	0.2	2	–
D	0.25	0.25	0.2	2	0.1
E	0.25	0.25	0.2	2	0.2

Woodstock, IL, USA), sodium carboxymethylcellulose viscosity grade: 500–2500 mPa s (Fluka AG, Buchs SG, Switzerland), polyethyleneglycol 400 (Parafarm, Buenos Aires, Argentina), and Asc16 (Sigma, Milwaukee, USA).

2.2. Formulation and preparation of the films

Procedure: Carbomer 934P (CB) and sodium carboxymethylcellulose (NaCMC) (1:1) were dispersed in water (50 ml) at 60–65 °C, by stirring and kept under vacuum. Nystatin (200 mg, particle size 300 μm), PEG400 and ASC16 were dispersed in water at 60–65 °C and then poured on polymeric dispersion under gentle stirring for 30 min. The gels were allowed to reach room temperature, and then placed into molds which were specially designed to produce a thin film (2 mm thickness) after water evaporation. The film forming gels contained in the molds were placed in an oven at 60–65 °C until constant weight was achieved (about 24 h). Dosage units were made by cutting film discs of 10.8 mm diameter. The moisture content of the films was less than 2% (assayed by TGA). The composition of the different films is detailed in Table 1.

2.3. "In vitro" mucoadhesion assay

Mucoadhesion was measured as the force required to pull a film out of a mucin gel layer (30%, w/w) using an adapted Jolly Balance (Facultad de Astronomía, Matemáticas y Física, Córdoba, Argentina) (Llabot et al., 2004). The films were fixed to a support with cyanoacrylate adhesive, then suspended from a spring and lowered until they just made contact with the surface of the mucin. A 50 μl of distilled water was placed between the film and mucin gel, and to produce adhesion a 20-*g* force was applied to the films for 30 s. Then, the platform was raised at 0.74 cm/s until the film was separated from the mucin. This break point represents the adhesive bond strength between these surfaces, which was expressed in N/cm^2. For each film composition, the assay was performed for five dosage units and then averaged.

2.4. X-ray powder diffraction (XRPD) of films

Films were analyzed using X-ray powder diffraction with a Rigaku Miniflex diffractometer equipped with the specific software Standard monitoring 3.2. The scan range was 1–30° 2θ/θ with a scan speed of 0.02° 2θ/s.

2.5. Morphological evaluation of the film

scanning electron microscopy (Siemens Autoscan, Germany) was employed. Samples were attached to pin-type mounts which had been previously covered with coated tape and sputtered with gold-palladium.

2.6. Nystatin release from the films

Nystatin release experiments were carried out using a device that simulates oral clearance as described in Spadaro et al. (2001), at 37 °C and 100 rpm with distilled water (10 ml). The films were fixed with a metal mesh, and then placed at the bottom of the vessel. Samples were withdrawn using a peristaltic pump Masterflex Dual-Channel Variable-Speed Compact C/L® Pumps (Barrington USA) (1 ml/min) and the collected fraction was deposited in a glass tube and measured at 306 nm with a UV–vis spectrophotometer (Shimadzu UV 160-A, Shimadzu Corporation, Kyoto, Japan).

3. Results and discussion

3.1. "In vitro" mucoadhesion

For comparative analysis of "in vitro" mucoadhesion, films A, C and D were selected to evaluate the effect of PEG400 and ASC16 on this property. The results are shown in Fig. 1.

The films formulated with the mixture CB:NaCMC (1:1) (film A) showed good "in vitro" mucoadhesion. However, the incorporation of PEG400 (film C) as well as PEG400 + ASC16 (film D) produced a significant increase in mucoadhesion that could have been associated to the plasticization of the polymeric network. This effect is in the line with the diffusion theory of bioadhesion (Chickering and Mathiowitz, 1999). It is thought that addition of plasticizers decreases the intra-chain polymeric interaction, thereby increasing the relaxation possibilities of the chains. Consequently, this higher flexibility can improve the inter-penetration and entanglement of bioadhesive polymer chains with mucous polymer (mucin), leading to the strengthening of mucoadhesive interactions.

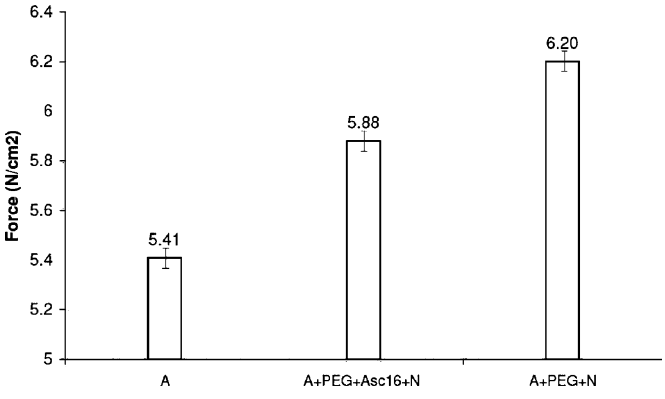

Fig. 1. "In vitro" mucoadhesion of films.

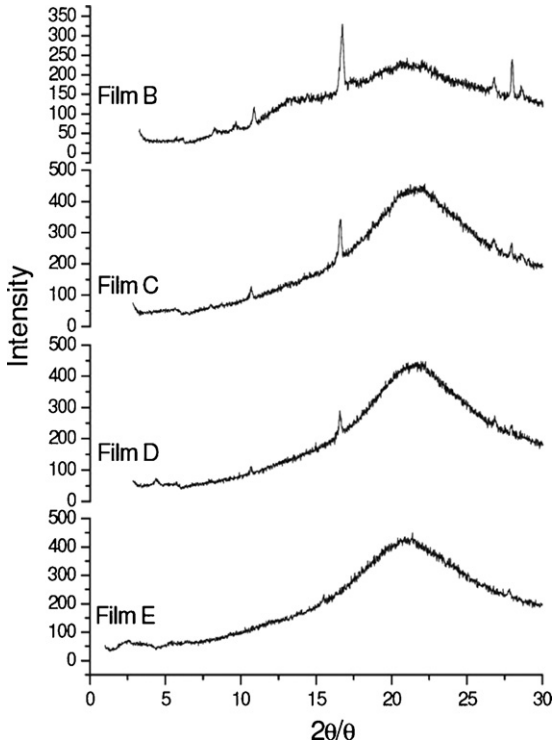

Fig. 2. XRPD of mucoadhesive films.

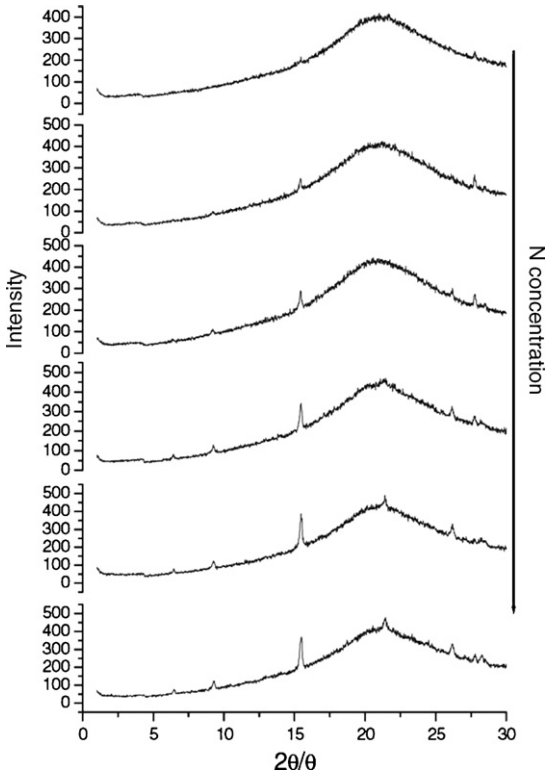

Fig. 3. XRPD of film C containing different concentrations of nystatin.

3.2. Incorporation of nystatin in the films

In the formulation of mucoadhesive films, the way in which the drug is incorporated may influence properties like (i) bioadhesion, (ii) mechanical strength, (iii) homogeneity and (iv) drug release. In the case of nystatin, which is a very low solubility drug (Michel, 1977), it is desirable to disperse the drug in a homogeneous way to prevent these kinds of drawbacks. However, in terms of the scope of this work we could not to discern if molecular nystatin was homogeneously dispersed or was incorporated into some kind of solid supramolecular aggregate resulting from interaction between the polymers, PEG400 and ASC16. In the context of this work, we will use the term "homogeneous dispersion" to define this phenomenon.

In this study, we used ASC16 for nystatin solubilization, and its subsequent incorporation in the semisolid aqueous dispersion of polymers produced gels that after casting yielded the films (see Section 2.2). ASC16 possesses surfactant properties and it is able to solubilize large amounts of hydrophobic drugs into supramolecular aggregates which have been described as coagels (Palma et al., 2002; Palma et al., 2003a,b,c). To evaluate how the drug remains in the films we used an XRPD analysis of such systems. The resulting diffractograms are shown in Figs. 2 and 3. The presence of nystatin crystals in the amorphous polymeric matrix produced several signals, the most intense being about $2\theta = 15.2^{\circ}$.

To evaluate if nystatin concentration in the film presents a linear relationship with the observed signal in XRPD diffractograms, we analyzed film C in which increasing and known amounts of nystatin were incorporated. The intensity of the signal was correlated to drug concentration and results are depicted in Fig. 4. The high level of correlation suggests that the signal disappearance is a quantitative indicator of the drug, which is homogeneously dispersed in the system. It is important to note that this methodology could be of analytical use in the determination of nystatin for this kind of pharmaceutical dosage form.

The intensity of the signal decreased as PEG400 and ASC16 were added to the films. Taking into account that the films are solid anhydrous systems, one may theorize that the disappearance of the nystatin signal implies that the drug is incorporated in an "amorphous solid solution" in the matrix.

Polymer carriers like CB and HPMC are particularly likely to form this kind of molecular dispersions as the polymer itself can be present in the form of an amorphous polymer chain network (Leuner and Dressman, 2000).

The effect of PEG400 and increasing concentrations of ASC16 presented an accumulative effect on the solid dispersion of nystatin. In this way, the crystallinity of nystatin, reflected in the peak intensity, decreases in the following sequence: film

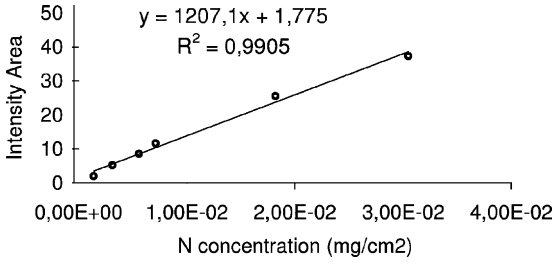

Fig. 4. Relationship of XRPD peak area vs. concentration of nystatin.

B > film C > film D > film E. In the case of film E, the signal is practically unobservable indicating that nystatin is almost totally dispersed in the matrix. On the other hand, it is predictable that PEG400 and ASC16 may influence the drug profile release from the films (see Section 3.4).

3.3. Morphological evaluation of the films

The sequence of SEM microphotographs ranging from film B to film E is shown in Fig. 5. If film B is considered, it can be clearly observed that the laminar structure of the film presents high crystallinity, resulting in a brittle film. The strong hydrophobic interactions between polymers could be the cause of this behavior, which is in agreement with film B forming gel compounded by CB:NaCMC (Llabot et al., 2006). Furthermore, on the surface of film B solid particles of nystatin are easily observed, indicating that the drug is totally insoluble in the film. This fact was detected in XRPD analysis of the film (Fig. 2).

The incorporation of PEG400 in the formulation (film C) produces a plasticization of the system giving rise to a less structured film, reflected in the smooth aspect of edge (Fig. 5c) and the globular structures that can be visualized on the surface. Fig. 5d also shows that nystatin particles are present on the film surface; in this way PEG400 only partially helps in the homogeneous dispersion of nystatin. When film C was analyzed through XRPD, the observed signal corresponding to nystatin (Fig. 2, Film C) had less intensity compared to film B. The addition of ASC16 (0.1 g, film D) produced a higher film plasticization

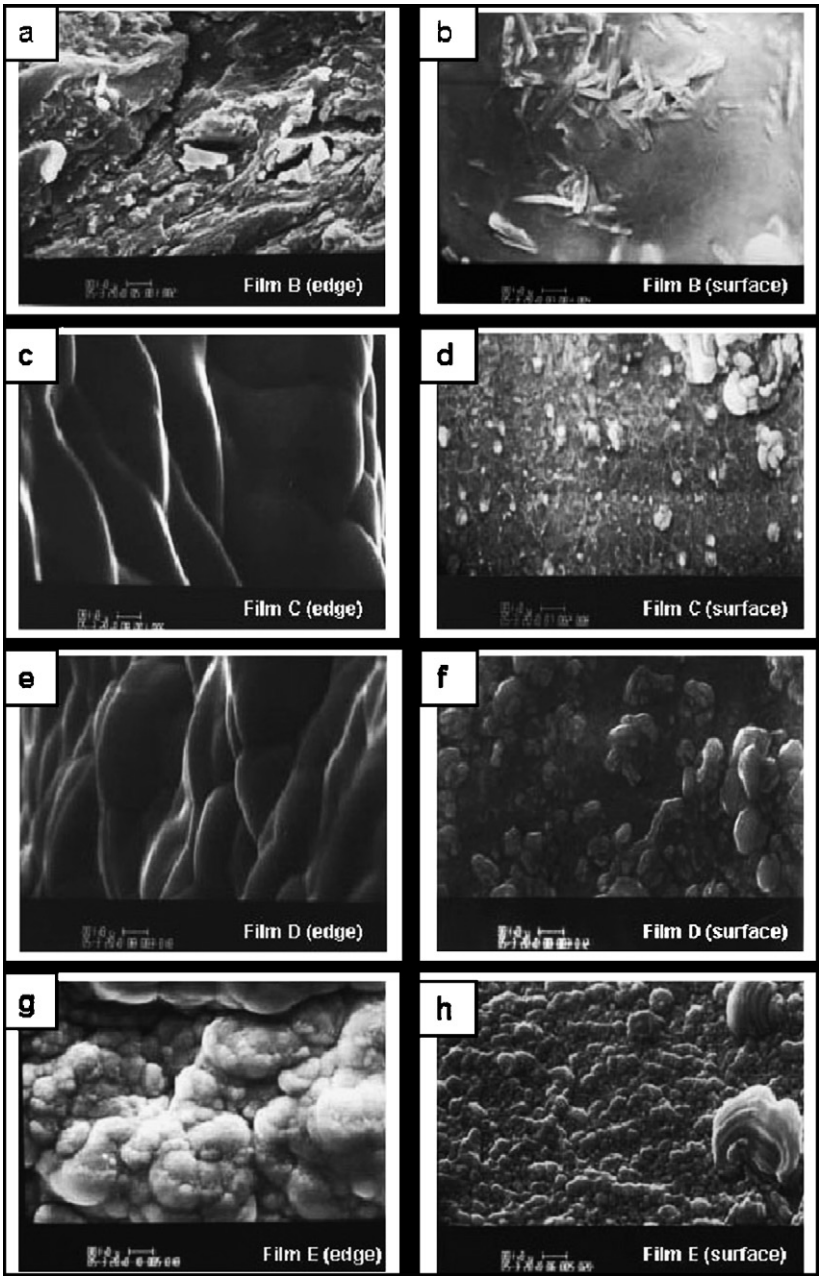

Fig. 5. SEM microphotographs of mucoadhesive gels (5000×).

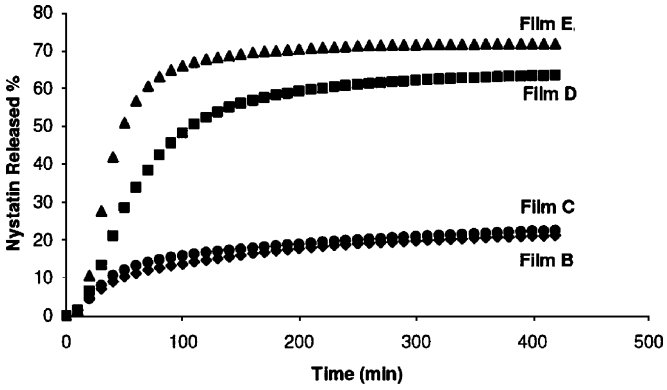

Fig. 6. Nystatin release from mucoadhesive films.

with consequent formation of globular structures. Nystatin was homogeneously dispersed in the gel although some solid particles were still seen on the surface. This observation is confirmed by an XRPD analysis (Fig. 2), where the drug signal was present but had a very low intensity. Finally, an increase in the ASC16 proportion of the film (0.2 g, film E) produced a film structure similar to film C, but with smaller globular units. However, nystatin was totally dispersed in a homogeneous way, being confirmed through the corresponding XPRD diffractograms. On the basis of these results, we postulated that nystatin is dispersed in the polymeric matrix, resulting in an amorphous solid mixture of components and drugs.

3.4. Nystatin release

It is known that the presence of surfactant in solid pharmaceutical dosage forms usually produces an increase in the rate of drug release, which depends of the drug and surfactant involved in each case. This effect is more prominent for films D and E than for films B and C, due to the incorporation of ASC16 in the films (Fig. 6). PEG400 has practically no effect on nystatin release since the rate is the same for films B and C.

Apparently, ASC16 exerts its effect through two principal mechanisms. The first could be favoring the homogeneous nystatin dispersion which is more advantageous for dissolution compared to the solid crystalline state. The second one could be a wetting-like effect, facilitating water penetration and consequent dissolution of the drug.

It is important to note that in the case of films D and E, nystatin release is relatively fast. Perhaps, a slower rate could be more convenient for mucoadhesive films, which have to be attached to the mucosa for at least 4 h. However, films D and E can be taken as starting points for optimization of these formulations. In order to have some certainty about the behavior of these systems, "in vivo", specific assays will be carried out in future research.

4. Conclusions

The incorporation of plasticizers (PEG400) and surfactant (ASC16) in the formulation of mucoadhesive films produced a significant improvement in the physical-mechanical properties and the nystatin release behavior. As these components were added to the systems, the films became more flexible, with the drug being homogeneously dispersed and the release rate increasing, compared to films made from a mixture of polymers alone. The XPRD methodology permits a quantitative evaluation into how the drug remains in the system. By means of SEM microscopy, the films changes produced as a consequence of the addition of PEG400 and ASC16 can be clearly observed, showing films losing their stratified structure and being replaced by more flexible and globular ones. Finally, it may be concluded that the strategy involving the addition of plasticizers, and in particular surfactants, can substantially improve the formulation of low solubility drugs in mucoadhesive films.

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